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Review

Succinate as a signaling molecule in the mediation of liver diseases

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ABSTRACT

Succinate, one of the intermediates of the tricarboxylic acid (TCA) cycle, plays an essential role in the metabolism of mitochondria and the production of energy, and is considered as a signaling molecule in metabolism as well as in initiation and progression of hepatic diseases. Of note, succinate activates a downstream signaling pathway through GPR91, and elicits a variety of intracellular responses, such as succinylation, production of reactive oxygen species (ROS), stabilization of hypoxia-inducible factor-1α (HIF-1α), and significant impact in cellular metabolism because of the pivotal role in the TCA cycle. Therefore, it is intriguing to deeply elucidate signaling mechanisms of succinate in hepatic fibrosis, metabolic reprogramming in inflammatory or immune responses, as well as carcinogenesis. This manuscript intends to review current understanding of succinate in mediating metabolism, inflammatory and immunologic reactions in liver diseases in order to establish molecular basis for the development of therapeutic strategies.

Introduction

Liver disease is a critical global public health issue with the greatly increased incidence and mortality. In 2010, the Global Burden of Disease Project evaluated that there were over 2 million deaths due to liver diseases, accounting for about 4 % of the annual deaths [1]. The number of patients suffering from liver diseases has continued to rise owing to alteration in living standards and irregular life style [2]. As one of emerging global pandemics, metabolic dysfunction-associated steatotic liver disease (MASLD) has reached nearly 25 % in its incidence over the world with more than 33 % in the US and 15 % in China [3,4]. As consequences of chronic liver disorders, cirrhosis and liver cancer represent the major causes of their mortality. Although advances in vaccines and therapies for hepatitis B/C viral infection, there is an urgent need to uncover the molecular mechanism of metabolic dysfunction-associated steatotic liver (MASL) and metabolic dysfunction-associated steatohepatitis (MASH), an active and progressive state of MASLD, and its evolution to fibrosis, cirrhosis, or even hepatocellular carcinoma (HCC) [4,5]. To date, there are no authoritative medications for the treatment of MASH, thus, elucidating the molecular mechanisms underlying the transition from MASL to MASH with fibrotic progression, additionally carcinogenic transformation from

Abbreviations: AC, adenylyl cyclase; α-KG, α-ketoglutarate; α-SMA, smooth muscle α-actin; cAMP, cyclic adenosine monophosphate; Col-I1, collagen type I; CTGF, connective tissue growth factor; DCs, dendritic cells; ECM, extracellular matrix; ERK, extracellular signal-regulated kinase; FAD, flavin adenine dinucleotide; FADH2, reduced flavin adenine dinucleotide; FFA, free fatty acids; GPCR, G protein-coupled receptor; HCC, hepatocellular carcinoma; HFCD-HF/G, high fat/calorie diet plus high fructose and glucose in drinking water; HIF-1α, hypoxia-inducible factor-1α; HSCs, hepatic stellate cells; IDH, isocitrate dehydrogenase; IFN, interferon; IP3, inositol triphosphate-3; I/R, ischemia and reperfusion; mDIC, mitochondrial dicarboxylate carrier; mROS, mitochondrial reactive oxygen species; MASH, metabolic dysfunction-associated steatohepatitis; MASL, metabolic dysfunction-associated steatotic liver; MASLD, metabolic dysfunction-associated steatotic liver disease; MCDAD, methionine and choline-deficient L-amino acid-defined; NADH, nicotinamide adenine dinucleotide hydride; NF-κB, nuclear factor-kappaB; OXPHO, oxidative phosphorylation; OXPHOS, oxidative phosphorylation system; PHDs, prolyl hydroxylases; PLC, phospholipase C; pVHL, von hippel-lindau gene product; ROS, reactive oxygen species; SDH, succinate dehydrogenase; SIRT, sirtuin; SUCNR1, succinate receptor-1; TCA, tricarboxylic acid; TGF-β, transforming growth factor-β; TIMP-1, tissue inhibitor of metalloproteinase-1; TNF-α, tumor necrosis factor-α; VHL, von hippel-lindau.

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MASH to HCC, is the key step towards the development of pharmacologic agents and preventive strategies.

As a major energy hub of cells, mitochondria are responsible for a variety of important metabolic processes, such as energy production [6], β-oxidation of fatty acids [7], gluconeogenesis [8] and detoxification metabolism [6], among which the tricarboxylic acid (TCA) cycle plays a pivotal role in metabolic and energy production [9]. Succinate is one of intermediate products in mitochondria TCA cycle, and has a vital role in mitochondrial energy generation [10]. Growing studies have focused on the functional actions of succinate except for the participation in energy metabolism, including signaling transmission, production of reactive oxygen species (ROS), stabilization and activation of hypoxia-inducible factor-1α (HIF-1α) [11–13], as well as the binding to one of G protein-coupled receptor (GPCR)s, GPR91 [14]. Metabolic reprogramming reflects cellular responses to critical changes in the microenvironment. When mitochondria are impaired due to various intracellular disturbances, the level of succinate is accumulated, in turn, the TCA cycle is disrupted [12]. Accumulated succinate may be converted into signaling molecules to regulate inflammatory or immunologic responses, and is critical for accelerating the transition from inflammation, fibrosis to cancer [15]. This manuscript intends to summarize recent findings with a primary focus on the mediation of succinate in signal transduction. Accumulating evidence points to that the mitochondrial succinate may become the new focus of interest in a variety of liver diseases, for instance, MASH with fibrotic progression, HCC and ischemia and reperfusion (I/R)-induced liver injury.

1. Succinate acts as a critical intermediator for energy metabolism and oxidant stress

1.1. Increased succinate and metabolic reprogramming

Succinate is one of the intermediate metabolites of TCA cycle in mitochondria and plays an indispensable role in metabolism. It is synthesized from α-ketoglutarate (α-KG), which is oxidized by the succinyl-CoA hydrolase, and further oxidized to fumarate with catalysis through succinate dehydrogenase (SDH) [16] (Fig. 1). As mentioned above, the TCA cycle is a core hub for energy production; therefore, succinate is involved in multiple metabolic pathways, including electron transport of the respiratory chain, phosphorylation of substrates, hemoglobin synthesis, etc. [16,17]. Succinate is generated and consumed upon status of energy production, and its levels vary with tissue energy consumption and any injuring processes [12]. Under a physiological condition, serum succinate levels are too low to exert specific effects. However, when intracellular succinate levels are markedly increased, it is transferred into the extracellular space and may act as a signaling molecule under certain circumstances [15]. Lipid toxicity or oxidative stress may lead to mitochondrial damage and affect activity of critical enzymes. On the other hand, disruption of TCA cycle hinders succinate oxidation to fumarate, resulting in an increase of succinate levels in MASH in which significant mitochondrial abnormalities have been identified due to lipotoxicity, oxidant stress and impaired mitophagy [18–20] (Fig. 1). In fact, these metabolic abnormalities are in sequential changes in metabolic programming, and represent cellular responses to necessary alterations of microenvironment [21,22]. For instance, mitochondria produce ATP through the oxidative phosphorylation system (OXPHOS) under normoxic conditions. Nicotinamide adenine dinucleotide hydride

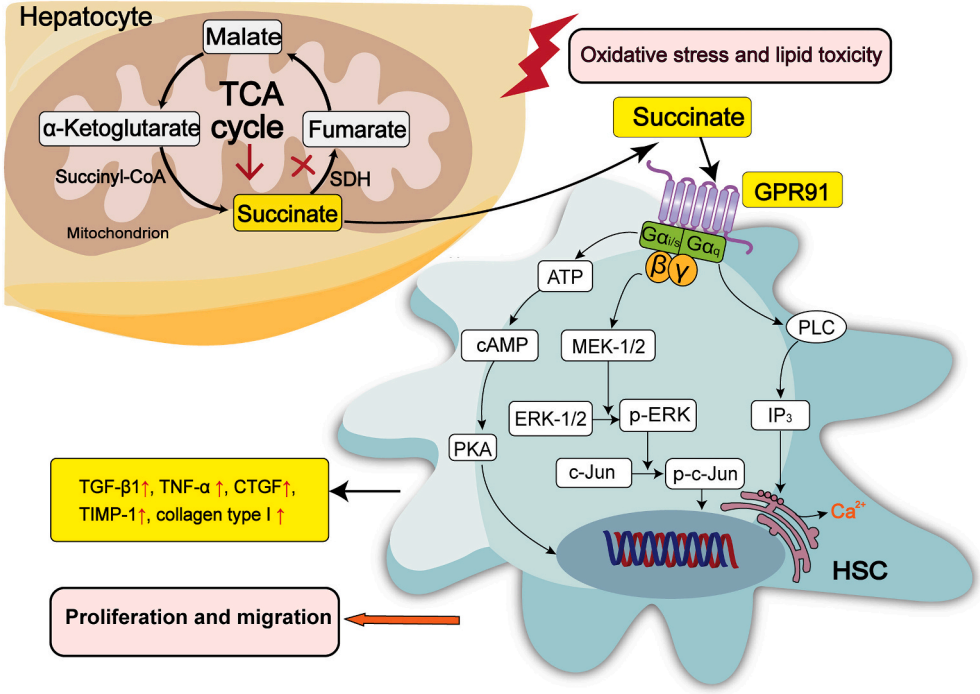

Fig. 1. Succinate is involved in the cross-talk between hepatocytes and HSCs. Succinate is synthesized from α-KG by the oxidation of succinyl-CoA hydrolase, and further oxidized to fumarate with catalysis by SDH. Lipid toxicity and oxidative stress may impair mitochondrial function and affect mitochondrial enzyme activity; in turn disruption of TCA cycle hinders oxidation from succinate to fumarate, leading to succinate accumulation. The coupling of succinate with GPR91 on the surface of HSCs results in a conformation change of GPR91, which activates the Gα subunit and leads to the separation of Gα subunit from Gβγ dimer. Then, Gα and Gβγ subunit further activate or inhibit the intracellular signaling cascades, such as, AC-cAMP, PLC-IP3, and elicit phosphorylation of downstream signaling molecules, such as ERK and c-Jun. These may further increase expression of several fibrogenic markers, such as TIMP-1, CTGF, TGF-β1, and Col-I1, causing the proliferation, migration, apoptosis, and inflammation of HSCs. Abbreviations used in this illustration: α-KG: α-ketoglutarate; SDH: succinate dehydrogenase; TCA: tricarboxylic acid; AC: adenylyl cyclase; PLC: phospholipase C; ERK: extracellular signal-regulated kinase; TIMP: tissue inhibitor of metalloproteinase; CTGF: connective tissue growth factor; TGF-β: transforming growth factor-β.

(NADH) and reduced flavin adenine dinucleotide (FADH2) are produced through plenty of reactions in the TCA cycle [23,24]. Conversely, when O_2 tension is low, cells start to generate ATP independently of OXPHOS through glycolysis [25]. Balanced metabolic pathways play a key role in the exchange of carbon among fatty acids, sugars, nucleic acids, and proteins; hence, metabolic capacity meets energy demand along with fluctuation in prevailing nutrient and oxygen levels. In addition to enhanced glycolysis, metabolic reprogramming may result in the following consequences: 1) interruption of the TCA cycle; 2) release of metabolites; 3) succinate as a substrate for succinylation of protein; 4) and fatty acid metabolism, amino acid metabolism, and ROS scavenging.

1.2. Succinylation of key proteins and enzymes for metabolic reprogramming

Succinylation modification is a crucial post-translational modification of proteins, and the process by which succinimidyl group donors (e.g. succinyl-CoA) covalently bind to lysine residues of substrate proteins, either enzymatically or non-enzymatically, modulates the status of TCA to cellular physiological activity [26]. The increase and decrease of succinylation are primarily regulated by succinyl donor and succinyl transferase/desuccinylation enzymes, which plays an important role in many kinds of metabolic reprogramming, such as increased synthesis of glycolysis and free fatty acids (FFA), decreased β-oxidation and protein synthesis, and ROS scavenging through regulating the activity of substrate protein or gene expression. Succinylation may affect the downstream protein and FFA synthesis by altering the rate of enzymes, including pyruvate dehydrogenase, isocitrate dehydrogenase (IDH), and SDH and various metabolic pathways, especially mitochondrial metabolic pathways [27]. In turn, the processes of mitochondrial β-oxidation of fatty acids to generate acetyl-CoA and NADH are affected by the changes of metabolic enzymes [28]. These metabolic reprogramming alterations may be participated in the occurrence and progression of different kinds of pathophysiological conditions including hepatic, metabolic, cardiovascular and neurologic disorders.

Succinylation occurs mainly in mitochondria and is involved in regulating activity of key enzymes in various metabolic processes, such as the operation of the TCA cycle, fatty acid β-oxidation, electron transport chain and active oxygen scavenging [26,29]. When glucose concentration increases, the level of mitochondrial succinylation is elevated [30]. When SDH is depleted, an increase in succinyl-CoA through the TCA cycle is boosted; at the same time, the lysine succinyl modification on the nucleosome is significantly enhanced [26,27]. Conversely, mitochondrial succinylation levels are significantly mitigated when glycolysis is reduced, and glutathione is depleted; on contrast, TCA cycle, electron transport and ATP synthesis are inhibited [31]. In fact, the occurrence of enhanced extra-mitochondrial succinylation suggests that in addition to succinyl-CoA, succinate or another succinyl-metabolite, such as fumarate, D-2-hydroxyglutarate, may affect the succinylation levels [27,32]. Most enzymes associated with the TCA cycle undergo succinylation modification, such as IDH, which has a crucial rate-limiting function [32], and in turn affects succinate levels along with rate of TCA cycle.

The counter-acting role of succinylation, so-called desuccinylation, especially in the progression of MASLD, has been studied focusing on sirtuin (SIRT) family [33]. The SIRT family is able to de-succinylate substrate proteins, thereby is critical for glycolysis, TCA cycle and mitochondrial function [34]. Mammals contain seven SIRT co-enzymes, which are named as SIRT-1 to 7. SIRT-5, as a key protein for desuccinylation, removes the succinyl-CoA group on the lysine residue of the SDH and pyruvate dehydrogenase complex, resulting in the desuccinylation of both enzymes within the TCA cycle [35,36]. In addition, SIRT5 affects 3-hydroxy-3-methylglutaryl-CoA synthase 2, a rate-limiting ketogenic enzyme, not only *in vitro* but also *in vivo* through de-succinylation [37]. It has been reported that both SIRT1 and SIRT4 have metabolic modulating impacts on the response of different dietary regiments to insulin resistance and MASLD in a rat model [38]. SIRT4 may be involved in regulating insulin secretion to address energy balance in metabolic processes, thereby preventing obesity and type 2 diabetes [38,39]. Undoubtedly, exploring the relationship between succinylation modification with various MASLD stages helps to delineate molecular mechanisms in the transition of MASL to MASH and development of insulin resistance, especially in those with metabolic syndrome or diabetes.

1.3. Activation and stabilization of HIF-1α

Growing evidence suggests that succinate is a signaling molecule that regulates the HIF-1α stabilization, which in turn triggers cytokine production, cell proliferation and angiogenesis [13]. Mechanistically, the HIF-1α stabilization is achieved by inhibiting activity of prolyl hydroxylases (PHDs) [40]. PHD enzymes, as Fe^{2+} and α-KG-dependent dioxygenase family, split molecular oxygen into hydroxylated HIF-1α [41]. HIF-1, a heterodimer consisting of HIF-α and HIF-β subunits, is also a nuclear transcription complex mediating the intracellular hypoxic response [42]. The HIF-α subunit is an oxygen-inducing component [43], and it has a vital part in mediating inflammatory signaling pathways through a lipopolysaccharide-Toll-like receptor-4 signaling axis and downstream events in various conditions of hypoxia and/or inflammation [13]. The *Von Hippel-Lindau* (*VHL*) gene product (pVHL), as an E3 ubiquitin ligase compound, is in the way of combining with oxygen dependency to HIF-α oxygen-dependence on degradation of domain structure [43]. Under normal physiological conditions, HIF-1α promotes its binding with the pVHL E3 ligase complex and acetylation of lysine residues, and results in the degradation of HIF-1α via the ubiquitin proteasome pathway. Whereas under hypoxia, succinate, may act as an antagonist, inhibits the activity of α-KG-dependent dioxygenase PHDs, resulting in the separation of pVHL from HIF-1α, thereby stabilizing HIF-

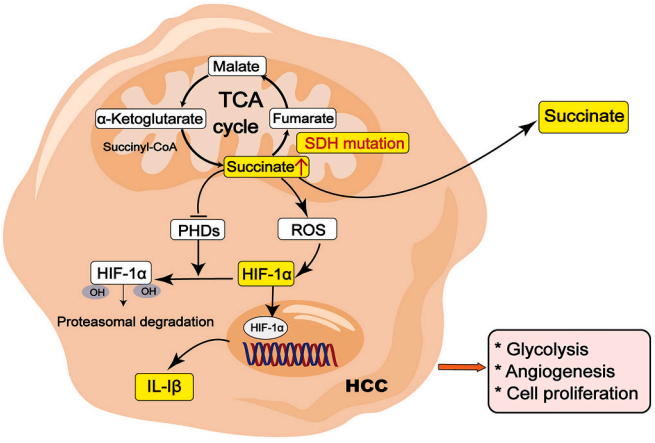

Fig. 2. Mutations of SDH increase the accumulation of succinate, causing metabolic reprogramming in HCC. PHDs activation induces hydroxylation of HIF-1α in normoxia, leading to ubiquitination and proteasomal degradation. SDH mutation in the tumor microenvironment results in a high concentration of succinate in the cytoplasm. Succinate has a structure similar to α-KG, both of them can interact and inhibit PHDs. Thus, the accumulation of succinate may result in the stabilization of HIF-1α, allowing its translocation into the nucleus to dimerize with HIF-1β and activate the transcription of its target genes. SDH mutation in the mitochondria also contributes to ROS production. This conversely results in HIF stabilization, GPR91 activation and downstream signaling cascades. The involvement and coordination of all these possible mechanisms increase the expression of specific genes, such as *IL-1β*, in promoting glycolysis, cell apoptosis, cell proliferation and migration in tumor tissues. Abbreviations used in this illustration: PHDs: prolyl hydroxylases; HIF: hypoxia-inducible factor; SDH: succinate dehydrogenase; α-KG: α-ketoglutarate; ROS: reactive oxygen species; TNF-α: tumor necrosis factor-α; *IL-1β*: Interleukin-1β.

1α, further regulating the elevation and reduction of downstream target gene expression, such as *IL-1β* and *TNF-α* [44,45] (Fig. 2). These genes govern an array of functions, including adaptive switches in metabolism, in addition to angiogenesis, cell proliferation, growth, and metastasis via epithelial-mesenchymal transition [46,47].

In addition to the direct inhibition of PHDs, succinate may indirectly aid in HIF-1α stabilization via enhancing oxidant stress [45]. Pharmacological inhibition of SDH-B has been illustrated to increase HIF-1α stabilization in an ROS-dependent manner [48] (Fig. 2). ROS affects HIF-1α by oxidizing Fe^{2+} to Fe^{3+}, which limits the activity of PHDs and thus indirectly enhances HIF-1α stabilization [49]. Therefore, identifying succinate as a danger signaling molecule for stabilizing HIF-1α may be a key step towards deep understanding of innate immunity in inflammatory and immunologic responses, angiogenesis and metabolic reprogramming in pathophysiologic and malignant conditions.

1.4. Succinate accumulation in donor organs

I/R are inevitable in organ transplantation, causing an accumulation of succinate and pronounced production of ROS in donor organs [50]. During ischemia, fumarate accumulates in the mitochondria due to the malate–aspartate shuttling and the purine nucleotide cycling, which is then reversed into succinate by SDH. While in reperfusion, SDH rapidly oxidizes accumulated succinate and produces large amounts of ROS in the mitochondria through reverse electron transfer of mitochondrial complex I (NADH: ubiquinone oxidoreductase) [51]. Collectively, this process causes mitochondria to produce a great amount of ROS responsible for donor organ damage. It was found that succinate accumulation in cytosol in a rat renal I/R-induced injury model was closely related to the increase of SDH-mediated H_2O_2 production, and it was most remarkably correlated to physiological succinate concentrations [51]. In addition, an increased SDH activity was closely related to the increased ROS generation, primarily within physiologic succinate concentrations [51]. Similarly, succinate accumulation through the production of fumarate and reversal of SDH was a common phenomenon of ischemia. In this context, succinate was believed to be a major factor of mitochondrial ROS generation during the phase of reperfusion following ischemic injury [50]. Thus, these findings suggested that succinate acts as an intermediate signaling molecule during I/R injury; hence, modulating mitochondrial succinate and ROS levels may prevent donor organ injury and improve organ quality, function, and survival after implantation. In summary, accumulation of succinate may be the initiator for oxidant stress of donor organs, and elucidating the critical rule of succinate may point to a new direction of pharmacologic intervention for improvement of quality and survival of donor organs.

2. SDH, an enzyme of metabolic switcher

SDH is an enzyme complex, which is not only a marker of mitochondrial protein, but also the center of the TCA cycle and the electron transport chain [52]. As the major molecular regulator of succinate concentrations, this enzyme allows succinate to be oxidized to fumarate in the TCA cycle and ubiquinone (coenzyme Q) to be reduced in the aerobic electron transfer chain to generate ATP via oxidative phosphorylation (OXPHO) [52]. Therefore, oxidation of succinate to fumarate reduces flavin adenine dinucleotide (FAD) and channels electrons to ubiquinone via SDH. In the pathological process of ischemia, hypoxia or hyperglycemia, SDH activity is inhibited, and intracellular succinate accumulates accordingly, may be released into extracellular space and act on other cell types.

2.1. SDH subunit mutations and succinate accumulation

SDH is the firstly-identified mitochondrial tumor suppressor gene encoded by the nucleus, consisting of six subunits encoding *SDH-A*, *SDH-B*, *SDH-C*, *SDH-D*, *SDH-AF1*, *SDH-AF2* [53,54]. Through *in vitro* and *in vivo* experiments, important role of *SDH* on the survival and metastasis of HCC and its molecular mechanism were investigated, as well as the expression level of four subunits of *SDH* (*SDH-A/B/C/D*) in HCC and its substantial clinical influence were discussed [55]. The major findings suggested that inactivation of *SDH* may substantially reduce expression of the four *SDH* subunits in HCC tissues [55]. Additionally, *SDH-C* knockdown facilitated hepatoma cell growth and metastasis by increasing ROS levels and subsequently stimulating nuclear factor-kappaB (NF-κB) pathway [55]. So far, it has been identified that genetic mutations in *SDH* occur in the progression of various cancers, including hepatic or renal malignancies and gastric or colon cancers [53]. Interestingly, it has been demonstrated that irregular accumulation of succinate in cytoplasm and extracellular fluid of malignant cells may be directly induced by *SDH-B* mutation [54,56]. These findings suggested that SDH, as a critical tumor-suppressive enzyme, may be essential for metabolic reprogramming, malignant behaviors, and serve as a molecular target of intervention.

2.2. Succinate receptor

GPR91, as one of the GPCRs, is assigned succinate receptor 1 (SUCNR1). It is distributed in diverse cell and tissue types, including hepatic stellate cells (HSCs), macrophages, dendritic cells (DCs) and platelets, as well as been involved in a multitude of pathophysiological processes, including, but not limited to the following examples: regulation of the immune system, for instance, many chemokines act through GPCRs; regulation of the autonomic nervous control, such as blood pressure, heart rate, and digestion [14]. GPR91 was considered to exist as an orphan receptor until succinate was denoted as its natural ligand [14]. Growing evidence demonstrated that GPR91 plays a vital regulatory role in a diversity of pathophysiologic processes, such as innate immunity, inflammation, allergic reactions, tissue repair, and oncogenesis [14,57–60].

Although four metabolites of TCA cycle, succinate, oxaloacetate, malate and α-KG, activate GPR91 to various extent, succinate remains the most potent ligand in eliciting the receptor activation [61,62]. Under conditions of metabolic stress, i.e. hyperglycemia or hypoxia, intracellular succinate levels may be inevitably to increase, and it is then released into extracellular space and body fluid, as well as in bloodstream, enabling to activate GPR91 and downstream signaling mechanism. This succinate-GRP91 signaling axis is propagated in a paracrine fashion, and is believed to be critical in hepatic fibrosis, hypertension, diabetic renal deficiency and retinopathy [58].

Indeed, the binding of particular activation signal may also initiate the activation of specific GPCR, and its coupling to and conformation changes of the G protein subunits, which regulate a plethora of cellular processes in response to diverse inputs. Thus, the downstream consequence of receptor activation relies on which G protein subunits are activated and generation of 2^{nd} messaging molecules, such as cyclic adenosine monophosphate (cAMP), inositol triphosphate 3 (IP3) and Ca^{2+} [63] (Fig. 1). For instance, the coupling of succinate with GPR91 on HSC surface results in a conformation change of GPR91, which activates the Gα subunit and elicits the separation of Gα subunit from Gβγ dimer. Then, the intracellular signaling cascades are further activated or inhibited by the Gα and Gβγ subunits, such as, the target of the Gαs and Gαi pathways is adenylyl cyclase (AC), which produces cAMP; and the object of the Gαq pathway is phospholipase C-β, and IP3 and diacylglycerol are produced by its catalytic membrane-bound phosphatidylinositol 4,5-bisphosphate. GPR91 may activate the mitogen-activated protein kinase kinase and the following pathways through Gβγ dimer [14,64–66]. It has been indicated that GPR91 exerts downstream signaling effects via two G-protein Gαi and Gαq pathways [67]. In addition, it is well known that GRP91 receptor activation elicited phosphorylation of downstream signaling molecules, like extracellular signal-regulated kinase (ERK) and c-Jun, which may further drive HSC activation as indicated by expression of smooth muscle α-actin (α-SMA)

and production of extracellular matrices, such as tissue inhibitor of metalloproteinase-1 (TIMP-1), as well as the release of fibrogenic cytokines, i.e. transforming growth factor-β (TGF-β) and connective tissue growth factor (CTGF) [68] (Fig. 1). In brief, succinate stimulates GPR91 in HSCs, a signaling pathway that triggers its coupling to various G protein subunits and downstream signaling mechanisms. In summary, elucidation the inter-association of GPR91 with diverse G protein subunits aids in characterizing the interactions of the 2nd messaging molecules across various cell kinds in response to changes in various metabolic and inflammatory microenvironments, uncovering critical pathways and molecules in different pathologic conditions.

2.3. GPR91 as a pharmacologic target for intervention

GPR91 is promising as a new pharmacologic target because it acts as a sensor for succinate accumulation and as part of a critical response during metabolic stress and hypoxia in a variety of conditions. With increasing understanding of the succinate-GPR91 axis in different pathophysiologic conditions, many studies are keen on determining therapeutic potential in specifically targeting this pathway through GPR91 antagonists or other strategies. Currently, several GPR91 antagonists have been synthesized for pharmacologic intervention. NF-56-EJ40, as a GPR91 antagonist, significantly interrupted succinate/IL-1β signal in macrophages and human umbilical vein endothelial cells, and then reduced inflammatory responses [69]. GPR91 may provide new insights into blocking succinate signaling to suppress inflammatory pathways in various organs [69]. Another GPR91 antagonist, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide, was found to alleviate the generation of collagen type I (Col-I1) in activated HSCs, thereby may have potential to improve liver fibrosis [57]. In summary, with the deep delineation of the succinate-GPR91 axis in liver disease, new pharmacotherapeutics may be available in the near future by the development of specific small molecule antagonists or antibodies against this signaling pathway.

3. Succinate and liver fibrosis

Hepatic fibrosis results from chronic damage induced by hepatitis B/C virus infection, alcohol abuse or MASH [70]. MASLD is essentially a chronically developmental disorder with histology ranging from simple steatosis to MASH. As the disease progresses, MASH may progress to progressive fibrosis and eventually to cirrhosis. Currently, developing therapeutic approaches aiming at repressing fibrosis or its reversion has been an urgent need of translational investigations [71]. However, up to now there have been no approved therapeutics for MASH-associated fibrosis in clinical practice.

3.1. Mechanisms of liver fibrosis

Activated HSCs and portal fibroblast-derived myofibroblasts are known to be the main cell types producing collagen in the liver [71], and activation of HSCs plays a central role for fibrogenesis under different modes of chronic injury [72]. After liver injury, HSCs undergo phenotypical changes, i.e. they trans-differentiate from static vitamin A-storing cells into proliferative myofibroblast-like cells [73]. The procedure is named as HSC activation with changes in morphology and gene expression profile, including increased proliferation, expression of new receptors, and accelerated synthesis of extracellular matrix (ECM) components, of which Col-I or III predominate [74], ultimately result in ECM deposition and scar formation in the liver with chronicity of injuring process.

3.2. Succinate as a signaling of HSC activation

Succinate, as an important factor in HSC activation, is considered to be an important signaling molecule that facilitates proliferation, migration, apoptosis, and excretion of pro-inflammatory cytokines, such as TNF-α or IL-6 from HSCs [58,75,76]. To determine whether succinate exerts an important role in mediating hepatic fibrosis, experimental studies have been conducted in mice by intraperitoneal succinate injection. The results showed noteworthy changes of the mice treated with succinate in contrast with the control group. The succinate-treated mice showed significantly increased levels of fibrotic markers, such as α-SMA and Col-I1, as well as increased production of inflammatory IL-6 and TNF-α cytokines [75]. Whereas, the question remains whether the administrated dose is pathophysiologically relevant in that study. Moreover, substantial evidence indicated that succinate levels are positively linked to metabolic diseases, like diabetes, hypertension or MASLD. For instance, decreased SDH enzyme activity was reported in baboons with liver fibrosis following chronic intake of ethanol [77]. Similarly, mitochondrial dysfunction was found in chronically ethanol-administered rats, and SDH activity was also reduced to some extent [78]. The inhibition of SDH activity resulted in an increase in succinate levels, and liver fibrogenesis was closely correlated with succinate levels in rats [78]. As known, inflammation, oxidative stress and ROS-mediated toxicity are the key elements in alcoholic liver injury and mitochondrial dysfunction. Therefore, it is rational to propose to target the succinate-GPR91 signaling axis as a therapeutic strategy to attenuate hepatic injury and to block fibrosis by controlling HSC growth and survival.

3.3. Succinate-GPR91 signaling in HSC activation

There is growing evidence that succinate levels were increased in MASH with fibrotic progression, in which GPR91 was highly expressed and activated [79,80]. In the liver, the GPR91 receptor is exclusively expressed in quiescent HSCs, and will be abnormally upregulated upon HSC activation during chronic injury [20]. The activation of HSCs was significantly increased after HSCs were treated with succinate, suggesting that succinate-GPR91 signal is one of key pathways contributing to HSC activation [76]. Succinate level and GPR91 activity were functionally correlated to fibrotic extent in a mouse model of MASLD [58]. Moreover, knocking down GPR91 expression by small interfering RNA abrogated HSC activation *in vitro*, suggesting that the receptor is crucial for HSC activation [58]. Activation of GPR91 receptor by succinate led to an increase in *α-SMA*, *pre-Col-Iα1* and *CTGF* mRNA levels of primary rat HSCs and HSC lines [20]. Further studies have shown that succinate stimulation led to increased expression of GPR91 receptor at the protein level in primary HSCs and HSC lines [20]. Furthermore, there was a marked relationship between elevated succinate levels and HSC activation in mouse models fed with a high fat/calorie diet plus high fructose and glucose in drinking water (HFCD-HF/G). More importantly, it was confirmed that increased GPR91 expression correlates with MASH severity and fibrosis progression in biopsy liver tissue from MASH patients who underwent clinical trials [20]. When isolated liver was in ischemia by blocking open blood flow to the portal vein, a release of succinate was increased, subsequently HSCs were activated in this experiment (may be through ROS production) [76]. The findings of above-mentioned studies indicate that the signal axis of succinate-GPR91 plays a significant role in HSC activation and even in the occurrence and progression of liver fibrosis, and succinate, to be a paracrine signaling molecule, may trigger HSC activation by ischemic liver cells. In summary, sole expression of GPR91 in HSCs confers obvious advantage in targeting this receptor for blocking hepatic fibrosis in chronic liver injuries, such as MASH. As mentioned above, serum and liver succinate levels were increased during cirrhosis. Thus, the activation of succinate-GPR91 axis activity renders a rationale target for pharmacologic intervention by GPR91 antagonists or specific antibodies.

As the regulatory function of the succinate-GPR91 axis in liver fibrosis is increasingly explored, targeting this signaling pathway to develop therapeutic interventions is of pharmaceutical relevance, hence

the development of small molecule antagonists or other strategy is in high priority. In one attempt, normal HSCs were cultured in a liver-equivalent gel to investigate how to reverse activated HSCs to a quiescent state, and it was reported when GPR91 was inhibited by specific antagonists, gene expression levels of *α-SMA* and *Col-I1* were suppressed accordingly in these cells [57]. Knocking-down of the GPR91 receptor by shRNA-carrying lentiviral vector in rats and human HSCs significantly led to a decrease in the protein level of the GPR91 receptor. The inhibition of the GPR91 receptor resulted in decreased α-SMA protein levels, mitigated phosphorylation levels of c-Jun and ERK-1/2 in the downstream signaling pathway, as well as reduced procollagen synthesis and CTGF production, confirming that inhibition of GPR91 receptor stimulation by succinate significantly reduced the HSC activation [20]. In a mouse model fed the methionine and choline-deficient L-amino acid-defined (MCDAD) diet, knockdown of *GPR91* gene expression via adeno-associated virus-mediated RNA showed that the mice with *GPR91* suppression significantly improved steatohepatitis and fibrosis [81]. To sum up, growing lines of evidence demonstrate that succinate and GPR91 hold great promise as effective targets for the prevention and/or therapy of MASLD with fibrotic progression. However, effective and safe small molecule compounds are still in the early stage of development, animal tests have not been carried out successfully, and the clinical translational remains to be determined.

4. Succinate as a promoting molecule in initiation and progression of liver cancer

Cancer is induced by a diversity of causes, including genetic mutations that drive cells to overmultiply [82]. Glycolysis is a typical characteristic of tumor cells in both hypoxic and normoxia states. Tumor cells rapidly produce a large amount of energy to meet their high biosynthetic and bioenergy needs, and foster their growth and development through the glycolysis pathway, which is called Warburg effect [83]. Recent studies have pointed out that in the development of cancer, abnormality in mitochondria is not a simple dysfunction; whereas disturbance of metabolic pathways is actually overwhelming [21,84]. During the metabolic reprogramming of tumor cells, levels of specific metabolites produced by mitochondria in tumor cells are higher than normal cells [83]. Cellular metabolite spectrum is considered to be an important indicator of pathophysiological states, and endogenous metabolites participate in the cellular regulation and other processes, such as immune homeostasis. When it is suppressed or compromised, oncogenic process may be initiated or boosted. Mitochondrial succinate did not receive much attention in relation to hepatic cancer until it was found to rise at cellular levels in some cancers and to be a potent signaling molecule of carcinogenic effects [85,86]. More studies have found that changes in the metabolic microenvironment, such as the increase or decrease of metabolite levels, may also be an essential trigger in the development of tumors [87]. The abnormal mitochondrial succinate accumulation may re-shape tumor microenvironment, driving cancer progression. In addition, abnormal transport of metabolic molecules between mitochondria and cytosol was found to play a role in tumor growth [21]. Succinate may directly participate in the development of tumor through the voltage-dependent anion channel in mitochondria [21]. Interestingly, it has been reported that mitochondrial dicarboxylate carrier (mDIC), which is mainly expressed in white adipose tissue, mediates the transport of succinate out of the mitochondrial matrix, improves obesity, and may be used as a dietary supplement to treat metabolic diseases [88]. The findings of these studies are concept-changing to the widely accepted research on succinate in liver diseases, and warrant further investigating regarding the role of succinate in specific *in vitro* and/or *in vivo* systems.

4.1. Hypoxia and HIF-1α in HCC

Hypoxia plays critical roles in tumor metabolism, increasing its initiation and progression [89]. Mitochondrial bioenergetic deficiency is a possible mechanism of the Warburg effect. HIF promotes the expression of various genes in response to hypoxia and is involved in the regulation of basic cellular metabolism such as tumorigenesis, angiogenesis, anaerobic metabolism and other biological mechanisms [90]. It was noted that succinate increased the stability of HIF-1α by inhibiting the activity of PHD in tumor cells, which enhanced the expression of certain specific genes that participated in glycolysis, angiogenesis, and cell proliferation, ultimately leading to tumor progression [12,91] (Fig. 2).

Exact mechanisms underlying how HIF-1α responds to hypoxia are unclear [83,92]. In the nucleus, the HIF-1 complex connects to hypoxic-responding progenitor cells on the hypoxic-response gene promoter region to recruit other transcription factors, and in turn triggers a series of hypoxia-adaptive responses in tissue cells [93], including the regulation of gene encoding glucose glycolysis enzymes, transporters, and proto-oncogene *c-myc*, etc. [93–95]. Therefore, during glycolysis, HIF-1α plays an essential role for transcriptional regulation in the process of metabolic reprogramming. The net consequence is to increase glucose metabolism, enhance cell proliferation, and boost angiogenesis, the critical steps for tumor progression as well as invasion. However, given that succinate inhibits PHDs by competing with ketoglutarate, it is pharmacologically possible to block the accumulation of succinate or to deliver ketoglutarate in sufficient quantities to counteract PHD inhibition and possibly induce tumor cell death [96].

4.2. SDH mutations and liver cancer

SDH is a tumor suppressor, and the mutation of *SDH* gene is accompanied with the increase of malignant behavior of tumor [91,96]. In tumors with *SDH* mutation, increased angiogenesis of activated HIF-1α or introduction of the following target genes, such as *vascular endothelial growth factor*, suggest that *SDH* mutations may induce tumorigenicity by activating the HIF-1α pathway. Indeed, *SDH* deficiency in cancer cells leads to irregular increase of succinate in cytoplasm and mitochondria, which inhibits PHDs and activates HIF-1α, providing a favorable environment for the growth of cancer cells [12,51,96] (Fig. 2).

SDH mutations are associated with enhanced mitochondrial ROS production [97]. Two studies confirmed that the mutations of *SDH-C* subunit led to increased levels of intracellular ROS stable states, leading to oxidative stress, DNA damage, genomic instability, and subsequent tumorigenesis [98,99]. Therefore, it is evident that the mutations of *SDH* subunits were accompanied with increased mitochondrial ROS production, indicating that mitochondrial respiration is severely disrupted. Additional studies reported that *SDH-C* subunit knockdown attenuated SDH activity as well as accelerated the proliferation and growth of hepatoma cells [55]. Mechanistically, the decrease of SDH activity induces the activation of downstream ROS/NF-κB pathway, which is involved in the regulation of cell proliferation and metastasis in liver cancer [55]. These findings validated that ROS is a major trigger of mutation-mediated SDH-C dysfunction leading to tumor progression, and demonstrated that inhibition of *SDH* mutations and hypoxic succinate accumulation are potential therapeutic strategies to prevent an inflammatory transition from chronic liver injury to hepatic malignancy and its further progression.

Although the mechanisms involved in tumorigenesis are complex, multiple layers of cross-talks have been proposed, and succinate may play a part in this complicated interplay. Firstly, succinate may act as a signaling molecule when it accumulates following mutations or loss-of-function in SDH [54]. Secondly, ROS generated in mitochondria due to mutated *SDH* may cause oxidative stress [50]. Thirdly, succinate secreted by cancer cells accumulates and enters the extracellular environment to activate GPR91 and downstream signaling mechanisms in tumor-associated macrophages, which are tightly associated with HCC progression [100]. Finally, it is worth to mention that succinate accumulation, in addition to acting as a signaling molecule, led to significant

DNA hypermethylation and apoptosis in a large number of cells, resulting in additional cytotoxic and carcinogenic effects [101,102]. In summary, the increase of succinate may be one of key factors in tumorigenesis; SDH could act as a tumor suppressor, and succinate can be regulated as a metabolite in tumors [51,54], for which, more research is warranted for unfolding underlying mechanisms.

5. Conclusion and prospects

Succinate, as an intermediate product of the TCA, occupies a key position in the metabolic process and accumulates in pathological conditions. Its accumulation affects TCA cycle, aerobic glycolysis, synthesis of fatty acids and lipids, and leads to metabolic reprogramming. Increased succinate levels may boost HIF-1α activation, ROS production, succinylation, post-translational modification and reprogrammed OXPHO in mitochondria because of its crucial role in the TCA cycle [12,44,103]. In addition to these intracellular effects, succinate is illustrated to act as an extracellular signaling molecule in an autocrine and paracrine mode to activate G protein-coupled GPR91 receptor, forming the succinate-GPR91 signaling axis and participating in the modulation of many metabolic downstream events in own or neighboring cells as summarized in the highlights (Table 1).

The pathogenesis of liver diseases is sophisticated, and different disorders share overlapping mechanisms. In MASH, fibrotic progression and liver cancer, elevation of succinate levels is closely correlated with the occurrence and progression in clinical settings [19,20,69,103]. However, both inflammation and malignancies share a similar hypoxia status with HIF-1α activation through increased succinate levels. Increased succinate levels may exert a tumorigenic effect in an inflammatory process. Therefore, succinate may be defined as a danger signaling molecule for better understanding of innate immunity in inflammatory diseases and cancer. It is intriguing that succinate may act as one of the key factors in macrophage polarization. However, the initiation and extent of polarization of macrophages vary from different etiologies, stages of pathologic processes and effectiveness of intervention. It is attempting to speculate that future studies may need to focus on delineating the importance of succinate as a key signaling intermediate for the regulation of immune function against cancer [104].

More importantly, succinate may induce several types of intracellular responses, such as HIF-1α stabilization, overproduction of mitochondrial ROS, as well as overall changes to metabolic homeostasis, due to its irreplaceable position in the TCA cycle. Furthermore, regulatory mechanisms of the succinate-GPR91 axis in various liver disorders were summarized together with potential antagonists, envisioning the future perspectives of the succinate-GPR91 axis as targeted cancer treatment. In speculation, selected small molecules that specifically target these factors or pathways may pharmacologically regulate the levels of succinate levels and thus hold potentials in treatment of liver diseases. Although this area of research is booming, there remain many obstacles since translation of potential small molecular compounds to clinical applications is highly costive with substantial risk of failure and takes years to decades of efforts for successful products.

In summary, succinate, as a critical signaling molecule in the mediation of liver and metabolic disorders, is expected to be a new therapeutic target due to its broad role in cellular metabolism and pathogenic processes. Selected antagonists are available for delineating signaling mechanisms of succinate-GPR91 axis in various tissue types and may be further optimized for preclinical or clinical translation.

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Table 1
Highlights.

- As a signaling molecule, succinate is critical for the initiation and progression of hepatic metabolic disorders
- Succinate elicits a variety of intracellular responses, such as HIF-1α stabilization, ROS production, succinylation and metabolic reprogramming
- Succinate-GPR91 signaling axis participates in the modulation of metabolic events in own or neighboring cells
- Mutations of succinate dehydrogenase (SDH) leads to accumulation of succinate and metabolic reprogramming in HCC
- Succinate and its receptor, GPR91, are potential therapeutic targets for the prevention and treatment of steatohepatitis, fibrosis and other disorders

Declaration of competing interest

All authors declare that no conflict of interest is associated with this work.

Data availability

Data will be made available on request.

References

[1] P. Byass, The global burden of liver disease: a challenge for methods and for public health, BMC Med. 12 (2014) 159.
[2] J. Xiao, F. Wang, N.K. Wong, J. He, R. Zhang, R. Sun, Y. Xu, Y. Liu, W. Li, K. Koike, W. He, H. You, Y. Miao, X. Liu, M. Meng, B. Gao, H. Wang, C. Li, Global liver disease burdens and research trends: analysis from a Chinese perspective, J. Hepatol. 71 (2019) 212–221.
[3] J.G. Fan, S.U. Kim, V.W. Wong, New trends on obesity and NAFLD in Asia, J. Hepatol. 67 (2017) 862–873.
[4] M.E. Rinella, J.V. Lazarus, V. Ratziu, S.M. Francque, A.J. Sanyal, F. Kanwal, D. Romero, M.F. Abdelmalek, Q.M. Anstee, J.P. Arab, M. Arrese, R. Bataller, U. Beuers, J. Boursier, E. Bugianesi, C. Byrne, G.E. Castro Narro, A. Chowdhury, H. Cortez-Pinto, D. Cryer, K. Cusi, M. El-Kassas, S. Klein, W. Eskridge, J. Fan, S. Gawrieh, C.D. Guy, S.A. Harrison, S.U. Kim, B. Koot, M. Korenjak, K. Kowdley, F. Lacaille, R. Loomba, R. Mitchell-Thain, T.R. Morgan, E. Powell, M. Roden, M. Romero-Gomez, M. Silva, S.P. Singh, S.C. Sookoian, C.W. Spearman, D. Tiniakos, L. Valenti, M.B. Vos, V.W. Wong, S. Xanthakos, Y. Yilmaz, Z. Younossi, A. Hobbs, M. Villota-Rivas, P.N. Newsome, N.N.c. group, A multisociety Delphi consensus statement on new fatty liver disease nomenclature, Hepatology (2023), https://doi.org/10.1097/HEP.0000000000000520.
[5] Y.Y. Yang, L. Xie, N.P. Zhang, D. Zhou, T.T. Liu, J. Wu, Updates on novel pharmacotherapeutics for the treatment of nonalcoholic steatohepatitis, Acta Pharmacol. Sin. 43 (2022) 1180–1190.
[6] B. Fromenty, M. Roden, Mitochondrial alterations in fatty liver diseases, J. Hepatol. 78 (2023) 415–429.
[7] K. Begriche, J. Massart, M.A. Robin, F. Bonnet, B. Fromenty, Mitochondrial adaptations and dysfunctions in nonalcoholic fatty liver disease, Hepatology 58 (2013) 1497–1507.
[8] A.K. Madiraju, D.M. Erion, Y. Rahimi, X.M. Zhang, D.T. Braddock, R.A. Albright, B.J. Prigaro, J.L. Wood, S. Bhanot, M.J. MacDonald, M.J. Jurczak, J.P. Camporez, H.Y. Lee, G.W. Cline, V.T. Samuel, R.G. Kibbey, G.I. Shulman, Metformin suppresses gluconeogenesis by inhibiting mitochondrial glycerophosphate dehydrogenase, Nature 510 (2014) 542–546.
[9] I. Martinez-Reyes, L.P. Diebold, H. Kong, M. Schieber, H. Huang, C.T. Hensley, M. M. Mehta, T. Wang, J.H. Santos, R. Woychik, E. Dufour, J.N. Spelbrink, S. E. Weinberg, Y. Zhao, R.J. DeBerardinis, N.S. Chandel, TCA cycle and mitochondrial membrane potential are necessary for diverse biological functions, Mol. Cell 61 (2016) 199–209.
[10] M.P. Murphy, L.A.J. O'Neill, Krebs cycle reimagined: the emerging roles of succinate and itaconate as signal transducers, Cell 174 (2018) 780–784.
[11] D. Sacks, B. Baxter, B.C.V. Campbell, J.S. Carpenter, C. Cognard, D. Dippel, M. Eesa, U. Fischer, K. Hausegger, J.A. Hirsch, M. Shazam Hussain, O. Jansen, M. V. Jayaraman, A.A. Khalessi, B.W. Kluck, S. Lavine, P.M. Meyers, S. Ramee, D. A. Rüfenacht, C.M. Schirmer, D. Vorwerk, Multisociety consensus quality improvement revised consensus statement for endovascular therapy of acute ischemic stroke, Int. J. Stroke 13 (2018) 612–632.
[12] M.A. Selak, S.M. Armour, E.D. MacKenzie, H. Boulahbel, D.G. Watson, K. D. Mansfield, Y. Pan, M.C. Simon, C.B. Thompson, E. Gottlieb, Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF-alpha prolyl hydroxylase, Cancer Cell 7 (2005) 77–85.
[13] G.M. Tannahill, A.M. Curtis, J. Adamik, E.M. Palsson-McDermott, A. F. McGettrick, G. Goel, C. Frezza, N.J. Bernard, B. Kelly, N.H. Foley, L. Zheng, A. Gardet, Z. Tong, S.S. Jany, S.C. Corr, M. Haneklaus, B.E. Caffrey, K. Pierce, S. Walmsley, F.C. Beasley, E. Cummins, V. Nizet, M. Whyte, C.T. Taylor, H. Lin, S. L. Masters, E. Gottlieb, V.P. Kelly, C. Clish, P.E. Auron, R.J. Xavier, L.A. O'Neill, Succinate is an inflammatory signal that induces IL-1beta through HIF-1alpha, Nature 496 (2013) 238–242.

[14] X. Li, L. Xie, X. Qu, B. Zhao, W. Fu, B. Wu, J. Wu, GPR91, a critical signaling mechanism in modulating pathophysiologic processes in chronic illnesses, FASEB J. 34 (2020) 13091–13105.
[15] R. Haas, D. Cucchi, J. Smith, V. Pucino, C.E. Macdougall, C. Mauro, Intermediates of metabolism: from bystanders to signalling molecules, Trends Biochem. Sci. 41 (2016) 460–471.
[16] H.A. Krebs, The history of the tricarboxylic acid cycle, Perspect. Biol. Med. 14 (1970) 154–170.
[17] M. Akram, Citric acid cycle and role of its intermediates in metabolism, Cell Biochem. Biophys. 68 (2014) 475–478.
[18] N.P. Zhang, X.J. Liu, L. Xie, X.Z. Shen, J. Wu, Impaired mitophagy triggers NLRP3 inflammasome activation during the progression from nonalcoholic fatty liver to nonalcoholic steatohepatitis, Lab. Invest. 99 (2019) 749–763.
[19] C. Demeilliers, C. Maisonneuve, A. Grodet, A. Mansouri, R. Nguyen, M. Tinel, P. Letteron, C. Degott, G. Feldmann, D. Pessayre, B. Fromenty, Impaired adaptive resynthesis and prolonged depletion of hepatic mitochondrial DNA after repeated alcohol binges in mice, Gastroenterology 123 (2002) 1278–1290.
[20] X.J. Liu, L. Xie, K. Du, C. Liu, N.P. Zhang, C.J. Gu, Y. Wang, M.F. Abdelmalek, W. Y. Dong, X.P. Liu, C. Niu, C. Yang, A.M. Diehl, J. Wu, Succinate-GPR-91 receptor signalling is responsible for nonalcoholic steatohepatitis-associated fibrosis: effects of DHA supplementation, Liver Int. 40 (2020) 830–843.
[21] S. Todisco, P. Convertini, V. Iacobazzi, V. Infantino, TCA cycle rewiring as emerging metabolic signature of hepatocellular carcinoma, Cancers (Basel) 12 (2019) 68.
[22] B. Kelly, L.A. O'Neill, Metabolic reprogramming in macrophages and dendritic cells in innate immunity, Cell Res. 25 (2015) 771–784.
[23] A. Viola, F. Munari, R. Sanchez-Rodriguez, T. Scolaro, A. Castegna, The metabolic signature of macrophage responses, Front. Immunol. 10 (2019) 1462.
[24] Y. Liu, R. Xu, H. Gu, E. Zhang, J. Qu, W. Cao, X. Huang, H. Yan, J. He, Z. Cai, Metabolic reprogramming in macrophage responses, Biomark Res. 9 (2021) 1.
[25] B. Kadenbach, Introduction to mitochondrial oxidative phosphorylation, Adv. Exp. Med. Biol. 748 (2012) 1–11.
[26] J. Smestad, L. Erber, Y. Chen, L.J. Maher 3rd, Chromatin succinylation correlates with active gene expression and is perturbed by defective TCA cycle metabolism, iScience 2 (2018) 63–75.
[27] Y. Yang, G.E. Gibson, Succinylation links metabolism to protein functions, Neurochem. Res. 44 (2019) 2346–2359.
[28] M.M. Adeva-Andany, N. Carneiro-Freire, M. Seco-Filgueira, C. Fernandez-Fernandez, D. Mourino-Bayolo, Mitochondrial beta-oxidation of saturated fatty acids in humans, Mitochondrion 46 (2019) 73–90.
[29] M. Alleyn, M. Breitzig, R. Lockey, N. Kolliputi, The dawn of succinylation: a posttranslational modification, Am. J. Physiol. Cell Physiol. 314 (2018) C228–C232.
[30] S.M. Bochmann, T. Spiess, P. Kotter, K.D. Entian, Synthesis and succinylation of subtilin-like lantibiotics are strongly influenced by glucose and transition state regulator AbrB, Appl. Environ. Microbiol. 81 (2015) 614–622.
[31] H. Chen, H. Xu, S. Potash, A. Starkov, V.V. Belousov, D.S. Bilan, T.T. Denton, G. E. Gibson, Mild metabolic perturbations alter succinylation of mitochondrial proteins, J. Neurosci. Res. 95 (2017) 2244–2252.
[32] B.T. Weinert, C. Scholz, S.A. Wagner, V. Iesmantavicius, D. Su, J.A. Daniel, C. Choudhary, Lysine succinylation is a frequently occurring modification in prokaryotes and eukaryotes and extensively overlaps with acetylation, Cell Rep. 4 (2013) 842–851.
[33] Y. Cheng, T. Hou, J. Ping, G. Chen, J. Chen, Quantitative succinylome analysis in the liver of non-alcoholic fatty liver disease rat model, Proteome Sci. 14 (2016) 3.
[34] B. Osborne, N.L. Bentley, M.K. Montgomery, N. Turner, The role of mitochondrial sirtuins in health and disease, Free Radic. Biol. Med. 100 (2016) 164–174.
[35] S. Kumar, D.B. Lombard, Functions of the sirtuin deacylase SIRT5 in normal physiology and pathobiology, Crit. Rev. Biochem. Mol. Biol. 53 (2018) 311–334.
[36] L.R. Bringman-Rodenbarger, A.H. Guo, C.A. Lyssiotis, D.B. Lombard, Emerging roles for SIRT5 in metabolism and cancer, Antioxid. Redox Signal. 28 (2018) 677–690.
[37] M.J. Rardin, W. He, Y. Nishida, J.C. Newman, C. Carrico, S.R. Danielson, A. Guo, P. Gut, A.K. Sahu, B. Li, R. Uppala, M. Fitch, T. Riiff, L. Zhu, J. Zhou, D. Mulhern, R.D. Stevens, O.R. Ilkayeva, C.B. Newgard, M.P. Jacobson, M. Hellerstein, E. S. Goetzman, B.W. Gibson, E. Verdin, SIRT5 regulates the mitochondrial lysine succinylome and metabolic networks, Cell Metab. 18 (2013) 920–933.
[38] Y.R. Chen, S.R. Fang, Y.C. Fu, X.H. Zhou, M.Y. Xu, W.C. Xu, Calorie restriction on insulin resistance and expression of SIRT1 and SIRT4 in rats, Biochem. Cell Biol. 88 (2010) 715–722.
[39] U. Mahlknecht, S. Voelter-Mahlknecht, Fluorescence in situ hybridization and chromosomal organization of the sirtuin 4 gene (Sirt4) in the mouse, Biochem. Biophys. Res. Commun. 382 (2009) 685–690.
[40] F. Yu, S.B. White, Q. Zhao, F.S. Lee, HIF-1alpha binding to VHL is regulated by stimulus-sensitive proline hydroxylation, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 9630–9635.
[41] M. Safran, W.G. Kaelin Jr., HIF hydroxylation and the mammalian oxygen-sensing pathway, J. Clin. Invest. 111 (2003) 779–783.
[42] G.L. Semenza, Regulation of mammalian O_2 homeostasis by hypoxia-inducible factor 1, Annu. Rev. Cell Dev. Biol. 15 (1999) 551–578.
[43] C.W. Pugh, P.J. Ratcliffe, Regulation of angiogenesis by hypoxia: role of the HIF system, Nat. Med. 9 (2003) 677–684.
[44] E.L. Mills, B. Kelly, A. Logan, A.S.H. Costa, M. Varma, C.E. Bryant, P. Tourlomousis, J.H.M. Dabritz, E. Gottlieb, I. Latorre, S.C. Corr, G. McManus, D. Ryan, H.T. Jacobs, M. Szibor, R.J. Xavier, T. Braun, C. Frezza, M.P. Murphy, L. A. O'Neill, Succinate dehydrogenase supports metabolic repurposing of mitochondria to drive inflammatory macrophages, Cell 167 (2016) 457–470 e413.
[45] E. Mills, L.A. O'Neill, Succinate: a metabolic signal in inflammation, Trends Cell Biol. 24 (2014) 313–320.
[46] A.A. Tirpe, D. Gulei, S.M. Ciortea, C. Crivii, I. Berindan-Neagoe, Hypoxia: overview on hypoxia-mediated mechanisms with a focus on the role of HIF genes, Int. J. Mol. Sci. 20 (2019) 6140.
[47] A. Zimna, M. Kurpisz, Hypoxia-inducible factor-1 in physiological and pathophysiological angiogenesis: applications and therapies, Biomed. Res. Int. 2015 (2015), 549412.
[48] R.D. Guzy, B. Sharma, E. Bell, N.S. Chandel, P.T. Schumacker, Loss of the SDHB, but not the SDHA, subunit of complex II triggers reactive oxygen species-dependent hypoxia-inducible factor activation and tumorigenesis, Mol. Cell. Biol. 28 (2008) 718–731.
[49] T. Kietzmann, A. Gorlach, Reactive oxygen species in the control of hypoxia-inducible factor-mediated gene expression, Semin. Cell Dev. Biol. 16 (2005) 474–486.
[50] E.T. Chouchani, V.R. Pell, E. Gaude, D. Aksentijevic, S.Y. Sundier, E.L. Robb, A. Logan, S.M. Nadtochiy, E.N.J. Ord, A.C. Smith, F. Eyassu, R. Shirley, C.H. Hu, A.J. Dare, A.M. James, S. Rogatti, R.C. Hartley, S. Eaton, A.S.H. Costa, P. S. Brookes, S.M. Davidson, M.R. Duchen, K. Saeb-Parsy, M.J. Shattock, A. J. Robinson, L.M. Work, C. Frezza, T. Krieg, M.P. Murphy, Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS, Nature 515 (2014) 431–435.
[51] J. Kamarauskaite, R. Baniene, D. Trumbeckas, A. Strazdauskas, S. Trumbeckaite, Increased succinate accumulation induces ROS generation in in vivo ischemia/reperfusion-affected rat kidney mitochondria, Biomed. Res. Int. 2020 (2020), 8855585.
[52] M. Rasheed, G. Tarjan, Succinate dehydrogenase complex: an updated review, Arch. Pathol. Lab. Med. 142 (2018) 1564–1570.
[53] L. Xia, H. Zhang, X. Wang, X. Zhang, K. Nie, The role of succinic acid metabolism in ovarian cancer, Front. Oncol. 11 (2021), 769196.
[54] C. Bardella, P.J. Pollard, I. Tomlinson, SDH mutations in cancer, Biochim. Biophys. Acta 2011 (1807) 1432–1443.
[55] J. Li, N. Liang, X. Long, J. Zhao, J. Yang, X. Du, T. Yang, P. Yuan, X. Huang, J. Zhang, X. He, J. Xing, SDHC-related deficiency of SDH complex activity promotes growth and metastasis of hepatocellular carcinoma via ROS/NFkappaB signaling, Cancer Lett. 461 (2019) 44–55.
[56] P.L. Tseng, W.H. Wu, T.H. Hu, C.W. Chen, H.C. Cheng, C.F. Li, W.H. Tsai, H. J. Tsai, M.C. Hsieh, J.H. Chuang, W.T. Chang, Decreased succinate dehydrogenase B in human hepatocellular carcinoma accelerates tumor malignancy by inducing the Warburg effect, Sci. Rep. 8 (2018) 3081.
[57] M. Sakai, T. Sumiyoshi, T. Aoyama, K. Urayama, R. Yoshimura, GPR91 antagonist and TGF-beta inhibitor suppressed collagen production of high glucose and succinate induced HSC activation, Biochem. Biophys. Res. Commun. 530 (2020) 362–366.
[58] Y.H. Li, S.H. Woo, D.H. Choi, E.H. Cho, Succinate causes alpha-SMA production through GPR91 activation in hepatic stellate cells, Biochem. Biophys. Res. Commun. 463 (2015) 853–858.
[59] C. Liang, J. Li, B. Tian, L. Tian, Y. Liu, J. Li, L. Xin, J. Wang, C. Fu, Z. Shi, J. Xia, Y. Liang, K. Wang, Foresight regarding drug candidates acting on the succinate-GPR91 signalling pathway for non-alcoholic steatohepatitis (NASH) treatment, Biomed. Pharmacother. 144 (2021), 112298.
[60] M. Trauelsen, T.K. Hiron, D. Lin, J.E. Petersen, B. Breton, A.S. Husted, S. A. Hjorth, A. Inoue, T.M. Frimurer, M. Bouvier, C.A. O'Callaghan, T.W. Schwartz, Extracellular succinate hyperpolarizes M2 macrophages through SUCNR1/GPR91-mediated Gq signaling, Cell Rep. 35 (2021), 109246.
[61] M. Trauelsen, E. Rexen Ulven, S.A. Hjorth, M. Brvar, C. Monaco, T.M. Frimurer, T. W. Schwartz, Receptor structure-based discovery of non-metabolite agonists for the succinate receptor GPR91, Mol. Metab. 6 (2017) 1585–1596.
[62] J. Gilissen, F. Jouret, B. Pirotte, J. Hanson, Insight into SUCNR1 (GPR91) structure and function, Pharmacol. Ther. 159 (2016) 56–65.
[63] D. Wootten, A. Christopoulos, M. Marti-Solano, M.M. Babu, P.M. Sexton, Mechanisms of signalling and biased agonism in G protein-coupled receptors, Nat. Rev. Mol. Cell Biol. 19 (2018) 638–653.
[64] L. Sundstrom, P.J. Greasley, S. Engberg, M. Wallander, E. Ryberg, Succinate receptor GPR91, a Gα(i) coupled receptor that increases intracellular calcium concentrations through PLCβ, FEBS Lett. 587 (2013) 2399–2404.
[65] J. Paek, M. Kalocsay, D.P. Staus, L. Wingler, R. Pascolutti, J.A. Paulo, S.P. Gygi, A. C. Kruse, Multidimensional tracking of GPCR signaling via peroxidase-catalyzed proximity labeling, Cell 169 (2017) 338–349 e311.
[66] W.K. Kroeze, M.F. Sassano, X.P. Huang, K. Lansu, J.D. McCorvy, P.M. Giguere, N. Sciaky, B.L. Roth, PRESTO-tango as an open-source resource for interrogation of the druggable human GPCRome, Nat. Struct. Mol. Biol. 22 (2015) 362–369.
[67] W. He, F.J. Miao, D.C. Lin, R.T. Schwandner, Z. Wang, J. Gao, J.L. Chen, H. Tian, L. Ling, Citric acid cycle intermediates as ligands for orphan G-protein-coupled receptors, Nature 429 (2004) 188–193.
[68] P. Crespo, N. Xu, W.F. Simonds, J.S. Gutkind, Ras-dependent activation of MAP kinase pathway mediated by G-protein beta gamma subunits, Nature 369 (1994) 418–420.
[69] J. Xu, Y. Zheng, Y. Zhao, Y. Zhang, H. Li, A. Zhang, X. Wang, W. Wang, Y. Hou, J. Wang, Succinate/IL-1β signaling axis promotes the inflammatory progression of endothelial and exacerbates atherosclerosis, Front. Immunol. 13 (2022), 817572.
[70] R. Bataller, D.A. Brenner, Liver fibrosis, J. Clin. Invest. 115 (2005) 209–218.

[71] F. Heymann, C. Trautwein, F. Tacke, Monocytes and macrophages as cellular targets in liver fibrosis, Inflamm. Allergy Drug Targets 8 (2009) 307–318.
[72] U.E. Lee, S.L. Friedman, Mechanisms of hepatic fibrogenesis, Best Pract. Res. Clin. Gastroenterol. 25 (2011) 195–206.
[73] Y.P. Han, Matrix metalloproteinases, the pros and cons, in liver fibrosis, J. Gastroenterol. Hepatol. 21 (Suppl. 3) (2006) S88–S91.
[74] H. Senoo, Y. Mezaki, M. Fujiwara, The stellate cell system (vitamin A-storing cell system), Anat. Sci. Int. 92 (2017) 387–455.
[75] S.Y. Park, C.T. Le, K.Y. Sung, D.H. Choi, E.H. Cho, Succinate induces hepatic fibrogenesis by promoting activation, proliferation, and migration, and inhibiting apoptosis of hepatic stellate cells, Biochem. Biophys. Res. Commun. 496 (2018) 673–678.
[76] P.R. Correa, E.A. Kruglov, M. Thompson, M.F. Leite, J.A. Dranoff, M. H. Nathanson, Succinate is a paracrine signal for liver damage, J. Hepatol. 47 (2007) 262–269.
[77] M. Arai, M.A. Leo, M. Nakano, E.R. Gordon, C.S. Lieber, Biochemical and morphological alterations of baboon hepatic mitochondria after chronic ethanol consumption, Hepatology 4 (1984) 165–174.
[78] V.D. Reddy, P. Padmavathi, N. Varadacharyulu, Emblica officinalis protects against alcohol-induced liver mitochondrial dysfunction in rats, J. Med. Food 12 (2009) 327–333.
[79] E.L. Mills, C. Harmon, M.P. Jedrychowski, H. Xiao, R. Garrity, N.V. Tran, G. A. Bradshaw, A. Fu, J. Szpyt, A. Reddy, H. Prendeville, N.N. Danial, S.P. Gygi, L. Lynch, E.T. Chouchani, UCP1 governs liver extracellular succinate and inflammatory pathogenesis, Nat. Metab. 3 (2021) 604–617.
[80] E.H. Cho, Succinate as a regulator of hepatic stellate cells in liver fibrosis, Front Endocrinol (Lausanne) 9 (2018) 455.
[81] Y.H. Li, D.H. Choi, E.H. Lee, S.R. Seo, S. Lee, E.H. Cho, Sirtuin 3 (SIRT3) regulates α-smooth muscle actin (α-SMA) production through the succinate dehydrogenase-G protein-coupled receptor 91 (GPR91) pathway in hepatic stellate cells, J. Biol. Chem. 291 (2016) 10277–10292.
[82] A.S. Nam, R. Chaligne, D.A. Landau, Integrating genetic and non-genetic determinants of cancer evolution by single-cell multi-omics, Nat. Rev. Genet. 22 (2021) 3–18.
[83] P. Vaupel, H. Schmidberger, A. Mayer, The Warburg effect: essential part of metabolic reprogramming and central contributor to cancer progression, Int. J. Radiat. Biol. 95 (2019) 912–919.
[84] A.S. Tan, J.W. Baty, L.F. Dong, A. Bezawork-Geleta, B. Endaya, J. Goodwin, M. Bajzikova, J. Kovarova, M. Peterka, B. Yan, E.A. Pesdar, M. Sobol, A. Filimonenko, S. Stuart, M. Vondrusova, K. Kluckova, K. Sachaphibulkij, J. Rohlena, P. Hozak, J. Truksa, D. Eccles, L.M. Haupt, L.R. Griffiths, J. Neuzil, M. V. Berridge, Mitochondrial genome acquisition restores respiratory function and tumorigenic potential of cancer cells without mitochondrial DNA, Cell Metab. 21 (2015) 81–94.
[85] X. Chen, B. Sunkel, M. Wang, S. Kang, T. Wang, J.N.R. Gnanaprakasam, L. Liu, T. A. Cassel, D.A. Scott, A.M. Munoz-Cabello, J. Lopez-Barneo, J. Yang, A.N. Lane, G. Xin, B.Z. Stanton, T.W. Fan, R. Wang, Succinate dehydrogenase/complex II is critical for metabolic and epigenetic regulation of T cell proliferation and inflammation, Sci. Immunol. 7 (2022) eabm8161.
[86] E. Dalla Pozza, I. Dando, R. Pacchiana, E. Liboi, M.T. Scupoli, M. Donadelli, M. Palmieri, Regulation of succinate dehydrogenase and role of succinate in cancer, Semin. Cell Dev. Biol. 98 (2020) 4–14.
[87] Z.E. Stine, Z.T. Schug, J.M. Salvino, C.V. Dang, Targeting cancer metabolism in the era of precision oncology, Nat. Rev. Drug Discov. 21 (2022) 141–162.
[88] Y.A. An, S. Chen, Y. Deng, Z.V. Wang, J.B. Funcke, M. Shah, B. Shan, R. Gordillo, J. Yoshino, S. Klein, C.M. Kusminski, P.E. Scherer, The mitochondrial dicarboxylate carrier prevents hepatic lipotoxicity by inhibiting white adipocyte lipolysis, J. Hepatol. 75 (2021) 387–399.
[89] G.L. Wang, B.H. Jiang, E.A. Rue, G.L. Semenza, Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O_2 tension, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 5510–5514.
[90] A. Pezzuto, E. Carico, Role of HIF-1 in cancer progression: novel insights, A Review, Curr. Mol. Med. 18 (2018) 343–351.
[91] W. Wu, S. Zhao, Metabolic changes in cancer: beyond the Warburg effect, Acta Biochim. Biophys. Sin. Shanghai 45 (2013) 18–26.
[92] A. Minchenko, I. Leshchinsky, I. Opentanova, N. Sang, V. Srinivas, V. Armstead, J. Caro, Hypoxia-inducible factor-1-mediated expression of the 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 (PFKFB3) gene. Its possible role in the Warburg effect, J. Biol. Chem. 277 (2002) 6183–6187.
[93] N. Al Hasawi, M.F. Alkandari, Y.A. Luqmani, Phosphofructokinase: a mediator of glycolytic flux in cancer progression, Crit. Rev. Oncol. Hematol. 92 (2014) 312–321.
[94] N. Goda, M. Kanai, Hypoxia-inducible factors and their roles in energy metabolism, Int. J. Hematol. 95 (2012) 457–463.
[95] J.W. Kim, I. Tchernyshyov, G.L. Semenza, C.V. Dang, HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia, Cell Metab. 3 (2006) 177–185.
[96] A. King, M.A. Selak, E. Gottlieb, Succinate dehydrogenase and fumarate hydratase: linking mitochondrial dysfunction and cancer, Oncogene 25 (2006) 4675–4682.
[97] G. Laurenti, D.A. Tennant, Isocitrate dehydrogenase (IDH), succinate dehydrogenase (SDH), fumarate hydratase (FH): three players for one phenotype in cancer? Biochem. Soc. Trans. 44 (2016) 1111–1116.
[98] B.G. Slane, N. Aykin-Burns, B.J. Smith, A.L. Kalen, P.C. Goswami, F.E. Domann, D.R. Spitz, Mutation of succinate dehydrogenase subunit C results in increased O_2.-, oxidative stress, and genomic instability, Cancer Res. 66 (2006) 7615–7620.
[99] T. Ishii, K. Yasuda, A. Akatsuka, O. Hino, P.S. Hartman, N. Ishii, A mutation in the SDHC gene of complex II increases oxidative stress, resulting in apoptosis and tumorigenesis, Cancer Res. 65 (2005) 203–209.
[100] J.Y. Wu, T.W. Huang, Y.T. Hsieh, Y.F. Wang, C.C. Yen, G.L. Lee, C.C. Yeh, Y. J. Peng, Y.Y. Kuo, H.T. Wen, H.C. Lin, C.W. Hsiao, K.K. Wu, H.J. Kung, Y.J. Hsu, C.C. Kuo, Cancer-derived succinate promotes macrophage polarization and cancer metastasis via succinate receptor, Mol. Cell 77 (2020) 213–227 e215.
[101] P.L. Sulkowski, S. Oeck, J. Dow, N.G. Economos, L. Mirfakhraie, Y. Liu, K. Noronha, X. Bao, J. Li, B.M. Shuch, M.C. King, R.S. Bindra, P.M. Glazer, Oncometabolites suppress DNA repair by disrupting local chromatin signalling, Nature 582 (2020) 586–591.
[102] W. Cui, Z. Huang, G.P. Pfeifer, Lack of major genome-wide DNA methylation changes in succinate-treated human epithelial cells, Int. J. Mol. Sci. 23 (2022).
[103] L. Tretter, A. Patocs, C. Chinopoulos, Succinate, an intermediate in metabolism, signal transduction, ROS, hypoxia, and tumorigenesis, Biochim Biophys Acta 2016 (1857) 1086–1101.
[104] S. Jiang, W. Yan, Succinate in the cancer-immune cycle, Cancer Lett. 390 (2017) 45–47.